

Z-DNA and other non-B-DNA structures are reversed to B-DNA by interaction with netropsin

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Received 10 January 1983

The interaction between the B-form specific ligands netropsin (Nt) and distamycin-3 (Dst-3) and DNA duplexes has been studied under conditions of salt concentration and low water activity that modify the polymer conformation into a non-B DNA form, putatively a Z-like form. Three polymers with strict alternating purine-pyrimidine sequences and GC content from 100–0% have been tested: poly(dG–dC)·poly(dG–dC), poly(dA–dC)·poly(dG–dT) and poly(dA–dT)·poly(dA–dT). The titrations by Nt and Dst-3 were followed by circular dichroism. Although specific binding of Nt to the Z-form of poly(dG–dC)·poly(dG–dC) does not occur, Nt reverses this Z structure to the B-type conformation; Dst-3 is, however, totally inefficient. The presumed non-B or Z-like structure of poly(dA–dC)·poly(dG–dT) is reversed to the B-form upon interaction with Nt; Dst-3 also induces this reversal but at higher ligand ratios. The modified B-structure of poly(dA–dT)·poly(dA–dT) in low water activity is efficiently reversed to the B-form by interaction with both Nt and Dst-3.

*Z-DNA–B-DNA reversal Netropsin Distamycin-3 Antibiotic, small groove-binding
Purine–pyrimidine alternation Polydeoxynucleotide–antibiotic pair DNA conformation*

1. INTRODUCTION

The small groove-binding antibiotics Nt and Dst-3 (fig.1) selectively interact with A·T base pairs in the right-handed B conformation of DNA [1–3]. These drugs show no significant binding affinity towards double-stranded RNA [1,4–6], A-type DNA [1,7] or most RNA–DNA hybrids [8]. Both drugs can also induce reversal from the A to B form of DNA [9–11]. We have shown that certain synthetic RNA–DNA and 2'-fluoro-2'-deoxy·DNA hybrids may also undergo the A to B-type transition upon interaction with Nt and Dst-3 [12,13]. The three polymers poly(dG–dC)·poly-

(dG–dC), poly(dA–dC)·poly(dG–dT) and poly(dA–dT)·poly(dA–dT) possess a purine-pyrimidine sequence which is a requisite for the possibility of the B to Z transition. Under various ionic and solvent conditions, all of these polymers can be turned to different degree, to non-B DNA forms. Poly(dG–dC)·poly(dG–dC) adopts the left-handed Z-form under high ionic strength condi-

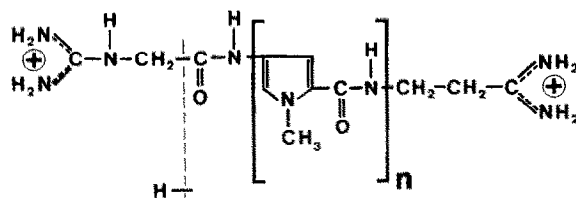


Fig.1. Chemical structure of netropsin (Nt; $n = 2$) and distamycin-3 (Dst-3; $n = 3$) lacking the left guanidinium group.

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Abbreviations: CD, circular dichroism; Nt, netropsin; Dst-3, distamycin-3

tions [14–21] or by interaction with spermine [15]. Poly(dA–dC)·poly(dG–dT) may also form a left-handed structure in fibres [15]. In solution, this latter polymer clearly differs from the B-form [22,23] although the existence of a Z-type structure has not yet been proven. Poly(dA–dT)·poly(dA–dT) exhibits a conformation different from the normal B-form in high CsF concentrations [24]; however, a Z-type conformation has been ruled out for this so-called X-form [25].

The Z-form of poly(dG–dC)·poly(dG–dC), as well as the non-B DNA forms of poly(dA–dC)·poly(dG–dT) and poly(dA–dT)·poly(dA–dT) in reduced water activity conditions are all characterised by long wavelength negative CD bands. The Z to B transition can be therefore conveniently monitored by CD. Here, we report the interaction of Nt and Dst-3 with the 3 polymers poly(dG–dC)·poly(dG–dC), poly(dA–dC)·poly(dG–dT) and poly(dA–dT)·poly(dA–dT) under solvent and ionic conditions favouring the non-B DNA conformations; i.e., the Z-form for poly(dG–dC)·poly(dG–dC) and the modified non-B forms for the two other polymers.

2. MATERIALS AND METHODS

Nt hydrochloride was a crystalline product [4] kindly donated by H. Thrum (Jena). Dst-3 was obtained from Boehringer, Mannheim. Extinction coefficients (in $M^{-1} \cdot cm^{-1}$) [26]: Nt, $\epsilon_{296} = 21\,500$; Dst-3, $\epsilon_{303} = 33\,000$. Poly(dG–dC)·poly(dG–dC), poly(dA–dC)·poly(dG–dT) and poly(dA–dT)·poly(dA–dT) were purchased from Boehringer (Mannheim); the extinction coefficients [27] were $\epsilon_{254} = 8400$, $\epsilon_{260} = 6500$ and $\epsilon_{258} = 6800 M^{-1} \cdot cm^{-1}$. The binding ratio, expressed as ligand/phosphate (r'), is used throughout. CD spectra were recorded on a Jobin-Yvon Dichrographe III using 1 cm cuvettes.

3. RESULTS

3.1. Poly(dG–dC)·poly(dG–dC)

This polymer adopts the Z-form in 60% ethanol plus 1.5 mM CsCl [22]. The interaction of Nt and Dst-3 in these conditions can be followed by the change in the CD spectrum and is shown in fig.2. Interaction of Nt appears to be cooperative; at $r' = 0.8$, when the titration reaches a plateau, the

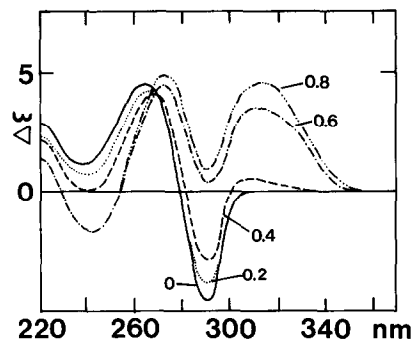


Fig.2. CD spectra of poly(dG–dC)·poly(dG–dC) in the Z-form in 1.5 mM CsCl, 60% ethanol with increasing amounts of Nt. The binding ratio r' is indicated.

CD band at 315 nm characteristic of the binding of Nt with a B-type structure is present. This suggests that a reversal from the Z to the B-type structure takes place under the action of Nt, even in the actual ionic and low water activity conditions that promotes the Z-form, due to the preferential interaction of Nt with the B-form of DNA. However, in contrast to Nt, Dst-3 does not induce any significant change in the CD spectrum, even at $r' = 0.9$. However, it should be noted that under low ionic conditions (such as 1 mM NaCl) where poly(dG–dC)·poly(dG–dC) exists as a B-type structure, Dst-3 interacts much better than Nt as judged from the intensity of the 320 nm band [28]. Increasing the ionic strength from 0.001–0.1 M nearly eliminates Dst-3 binding to B-form poly(dG–dC)·poly(dG–dC) (not shown).

On the other hand, Nt did not interact with poly(br^8dG –dC)·poly(br^8dG –dC) which is always in the Z form [29] and whose low salt CD spectrum is close to that of poly(dG–dC)·poly(dG–dC) in 60% ethanol (fig.2, $r' = 0$). No amount of Nt would reverse its spectrum to that of the B-form.

3.2. Poly(dA–dC)·poly(dG–dT)

In 60% ethanolic solution plus 1.5 mM CsCl and 0.02 mM Ca^{2+} , an intense, negative, long-wavelength CD band characterizes the non-B DNA conformation of this duplex [22]. Fig.3 shows the ability of Nt to interact with poly(dA–dC)·poly(dG–dT) in these conditions. Addition of Nt causes a gradual disappearance of the negative CD band at 278 nm; this effect is paralleled by the appearance of the positive CD band around 315 nm,

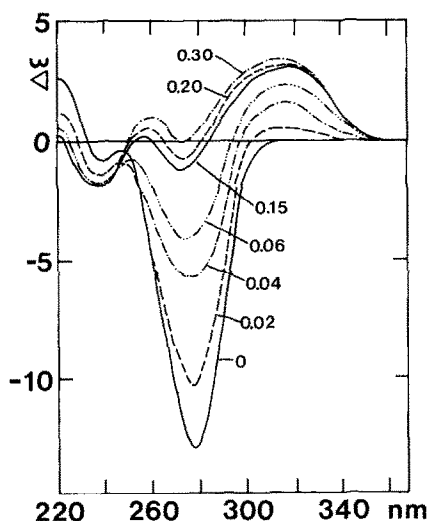


Fig.3. CD spectra of poly(dA-dC)·poly(dG-dT) in 1.5 mM CsCl, 60% ethanol, 0.02 mM Ca^{2+} with increasing amounts of Nt. Binding ratios r' are indicated.

characteristic of the binding of Nt to the B-form. Contrary to poly(dG-dC)·poly(dG-dC), Dst-3 lowers also the negative band at 278 nm and induces the positive one at 320 nm; this requires, however, higher r' values than Nt. The ligand-induced conformational reversal is different in 6 M CsCl, a condition which also promotes the appearance of a non-B DNA conformation for poly(dA-dC)·poly(dG-dT) [22]. In 6 M CsCl, Nt does not interact with this duplex, in agreement with our findings that high [salt] dissociates Nt from poly(dA-dC)·poly(dG-dT) [30], whereas Dst-3 and Dst-5 show pronounced binding (not shown).

3.3. Poly(dA-dT)·poly(dA-dT)

The binding of Nt to this polymer in 60% ethanol is shown in fig.4. Under these conditions of decreased water activity this alternating duplex adopts also a modified conformation which is not a Z-type DNA and which differs from that at low ionic strength by an intense, negative, long-wavelength Cotton effect [24,25]. The progressive lowering of the negative CD band at 278 nm indicates that Nt reverses the low water form of poly(dA-dT)·poly(dA-dT) to the B-form as a consequence of its stabilizing effect on the B-conformation. This reversal takes place within a

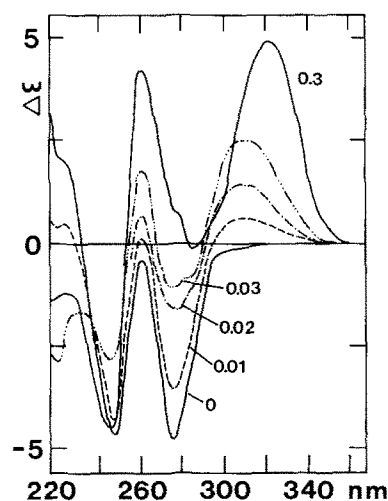


Fig.4. CD spectra of poly(dA-dT)·poly(dA-dT) in 1.5 mM CsCl, 60% ethanol, with increasing amounts of Nt. Binding ratios r' are indicated.

narrow range of ligand concentration up to $r' = 0.1$. In contrast with the two previous duplexes, Dst-3 is as efficient as Nt to induce the reversion to the B-form (CD spectra not shown).

4. DISCUSSION

Nt and Dst-3 are DNA binding ligands specific for the B-conformation [1-3]. These results show that these antibiotics can also reverse some alternating purine-pyrimidine duplexes to the B-form even under conditions of high [salt] and low water activity that induces a non-B form or the Z-form [18-22]. A reversal of the A to the B form has already been observed in DNA [11], and in some ribo-deoxy and 2'-fluoro-containing hybrids [12,13]. To compare easily the effects of Nt and Dst-3 on the 3 polymer duplexes studied one can examine the CD titrations monitored at two characteristic wavelengths (fig.5). The first positive CD peak at 315 and 328 nm for Nt and Dst-3, respectively, reflects the binding of the ligand to the duplex. The second wavelength corresponds to the negative peak around 275 to 288 nm which is characteristic for the Z form or at least of a non-standard B form [22-25] of poly(dA-dT)·poly(dA-dT) and poly(dA-dC)·poly(dG-dT). This wavelength allows one to

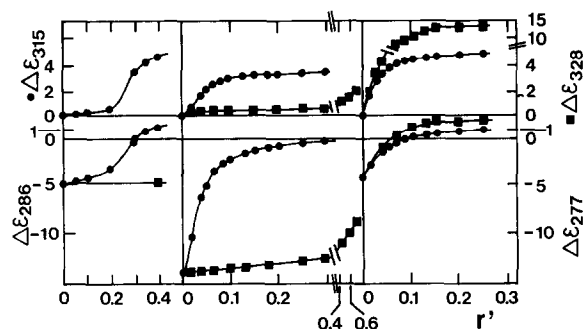


Fig.5. CD titrations of poly(dG-dC)·poly(dG-dC) (left), poly(dA-dC)·poly(dG-dT) (center) and poly(dA-dT)·poly(dA-dT) (right) with Nt (●) and Dst-3 (■). Experimental conditions are those of fig.2-4.

monitor the reversion of the non-B to the B-type CD spectrum (fig.5).

The following conclusions can be drawn:

- (1) For a given antibiotic-polymer pair the titrations at the two wavelengths are always parallel. This strongly indicates that the binding of the antibiotic and the reversion to the B-form are concomitant and that essentially no free B-type polymer is present as an intermediate.
- (2) An apparent inverse relationship exists between Nt binding and the ease of formation of non-B-like structures in alternating purine-pyrimidine duplexes. It is therefore not surprising that the easier a polymer binds Nt in low [salt], the more difficult it will be to turn it into the non-B forms and the more easily it will return to a B-form under the action of Nt.
- (3) In the search for the mechanism of the reversion from the non-B like forms to the B forms by Nt one has to take into account the large differences in the action of Nt and Dst-3. The efficiency of Dst-3 to reverse non-B forms decreases from poly(dA-dT)·poly(dA-dT) to poly(dA-dC)·poly(dG-dT). Dst-3 is totally inefficient on poly(dG-dC)·poly(dG-dC) in the Z-form (60% ethanol, 1.5 mM CsCl), while it binds efficiently in 0.1 M NaCl to this polymer in the B-form.

Both Nt and Dst-3 are non-chiral molecules containing a plane of symmetry. Absence of binding

of these molecules to the non-B-like or Z-form should therefore not be searched in the difference of handedness of such helices, but rather in the differences in their geometries. Nt binds to O² of pyrimidines or N³ of purines in B-DNA, as well as to the phosphate sites [1-9]. The minor groove, where the distance between phosphate groups on opposite strands is ~12 Å, is most favourable for the interaction of both oligopeptides. In Z-DNA, however, the phosphate groups are much closer to each other [14-16,31] and N³ of the purines is inaccessible because of the *syn* conformation of the guanosine residues, while the pyrimidine O² nearly touch the helix axis. The single groove of Z-DNA is very deep [14-16,31] so that interactions with phosphates and contacts with the bases are highly unfavoured. Nt thus induces a conversion to the B-form to which it binds with high efficiency.

It has been suggested [13] that Nt could replace one of the two water shells of the hydration spine in the small groove of the B-structure of A·T (and I·C) polymers [32]. The efficient reversal of poly(dA-dT)·poly(dA-dT) to the B-form by Nt and Dst-3 is probably accompanied by the replacement of the water spine by the antibiotics even under conditions of low water activity which do not favour the B-form. The absence of such a water spine in G·C pairs [32] greatly reduces binding, besides the steric hindrance by the 2-amino group of guanosine.

Our data also agree with recent calculations on different DNA conformations by the group of Pullman [33-35] which showed that the electrostatic potential is highest in the minor groove of B-DNA and represents an additional important factor in the binding ability of Nt and Dst-3. Interaction of both drugs with Z-DNA is electrostatically unfavoured [35].

In [21], reversal of the Z to B forms was observed under the action of intercalating drugs. Their finding that Dst-3 was ineffective on the Z-form of poly(dG-dC)·poly(dG-dC) agrees with these data in ethanolic solutions (fig.2,5).

Two points remain unclear and call for a deeper analysis of the Z to B reversal: the mechanism of the reversion by Nt and the inability of Dst-3 to reverse the Z-form of poly(dG-dC)·poly(dG-dC), although the latter antibiotic binds better to the B-form than Nt [28]. Work is under way to clarify these questions.

We have been informed recently by Professor C.W. Schmid (Univ. California, Davis) that poly(dA-dC)·poly(dG-dT) from Boehringer contained sequences different from those expected. This result, however, does not change our conclusion that a non-B DNA is returned to a B-type DNA under the influence of Nt and Dst-3. Whether this non-B form is in fact a Z-DNA (or any other poly(dA-dC)·poly(dG-dT) sample for that matter) will have to be resolved by other techniques and is under investigation.

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